Variability among *Cryptosporidium parvum* genotype 1 and 2 immunodominant surface glycoproteins

K. SESTAK[1], L. A. WARD[2], A. SHEORAN[1], X. FENG[1], D. E. AKIYOSHI[1], H. D. WARD[1,3] & S. TZIPORI[1]

[1]Department of Biomedical Sciences, Tufts University School of Veterinary Medicine, North Grafton, MA, [2]Food Animal Health Research and Development Program, The Ohio State University, Wooster, OH and [3]Division of Geographic Medicine and Infectious Diseases, New England Medical Center, Boston, MA, USA

SUMMARY

Published genomic differences between Cryptosporidium parvum *genotype 1 (human-derived) and genotype 2 (animal and human-derived) isolates suggest that these may belong to two distinct species. This is of significant interest since genotype 1 isolates are associated with sporadic cases of human cryptosporidiosis in 30–40% of cases in contrast to 60–70% of cases caused by genotype 2. The lower genetic sequence similarity between genotype 1 and 2 surface glycoproteins (gp40/15) suggests that antigenic differences should also occur, a feature that was investigated in this study. Using immune and convalescent serum samples from gnotobiotic piglets previously inoculated with genotype 1 and 2 isolates, we demonstrated that* C. parvum *gp15 was immunodominant for both genotype 1 and 2 isolates. Lower genetic sequence similarity between genotype 1 and 2 Cpgp40/15 did correspond to gp15 protein differences as detected by Western blot. Moreover, we confirmed that gp15 contains epitopes that are also immunodominant. Deglycosylation of* C. parvum *proteins resulted in decreased ability of gp15, gp23 and gp900 to react with homologous polyclonal antibodies, suggesting that these proteins also express carbohydrate epitopes. Taken together, our data suggest that there is a high phenotypic variability between* C. parvum *genotype 1 and 2 isolates at the level of gp15. We contemplate that gp15 surface glycoprotein plays an important role in the biology of* C. parvum *as a potent inducer of immune response and a possible virulence factor.*

Keywords *Cryptosporidium, protozoa, Apicomplexa, chronic diarrhoea, Cpgp40/15, AIDS*

INTRODUCTION

Cryptosporidium parvum is a protozoan parasite belonging to the phylum Apicomplexa, subclass Coccidia. *C. parvum* causes a self-limiting infection of the small intestine in immunocompetent humans or animals, but it also can be persistent (1) and life threatening in immunocompromised individuals, particularly those with AIDS (2). Even though *C. parvum* was described in 1907 (3), it was not recognized as a pathogen of mammals until 1971 when the infection was linked to calf diarrhoea (4). Chronic cryptosporidiosis became recognized with the emergence of the human immunodeficiency virus (HIV) and AIDS (5). Human cryptosporidiosis is attributed to two major genotypes (6–8), of which type 1 is found exclusively in humans, while type 2 is zoonotic and found in other mammals, including humans. A recent analysis of some 450 isolates from children with diarrhoea in Uganda, collected over 15 months, showed that 74% were of type 1 while only 19% were type 2 (manuscript in preparation). In contrast, an analysis of 1705 sporadic cases from the UK, showed type 2 to be predominant in 62% of cases compared to 38% of type 1 (9,10). There is a paucity of information regarding type 1 since only type 2 isolates were extensively studied utilizing *in vivo* or *in vitro* models. The recent successful transmission and serial propagation of human derived *C. parvum* type 1 isolates in gnotobiotic piglets by our group (11) offers new opportunities to examine human type 1 isolates. In contrast to severe clinical symptoms and a longer period of shedding associated with type 2 infection in gnotobiotic piglets, animals inoculated with type 1 exhibit less severe cryptosporidiosis (11). The genotypic heterogeneity among *C. parvum* isolates has not been correlated so far with phenotypic, traits including virulence, cell tropism or the nature of the immune response. Several structural surface glycoproteins (gp) have been identified in *C. parvum* sporozoites. They are thought to mediate the initial parasite attachment and/or invasion to the host cell (12–15), as specific monoclonal

antibodies (Mab) against them could inhibit attachment and invasion (13,15,16). More recent studies with monoclonal antibodies to sporozoite surface proteins revealed the presence of glycosylated epitope residues present in the two oocyst-/sporozoite-surface proteins of 40 and 900 kDa (12). The glycosylated nature of these proteins (gp) suggested the possibility of facilitating parasite to host-cell attachment through the carbohydrate epitopes. The gene *Cpgp40/15* (also named gp15/40/60 or Cp17), encoding a precursor protein which is cleaved to yield polypeptides gp40 (the 40 kDa glycoprotein referred to above) as well as gp15, has been cloned and sequenced (13–15). This gene exhibits a high level of polymorphism among *C. parvum* type 1 versus type isolates (14). The level of *Cpgp40/15* genetic and amino acid diversity suggests that antigen differences should also occur, a feature that was investigated in our study.

MATERIALS AND METHODS

C. parvum

Prototype strains of type 1 (TU502) and type 2 (GCH1), with distinct genetic differences at multiple loci as detected by microsatellite analysis (17), were used for animal inoculations, immunizations and as enzyme-linked immunosorbent assay (ELISA)/Western blot antigens. Oocysts were purified from faeces of infected calves as previously described (18). Briefly, the procedure involved homogenization and filtration of faeces in distilled water, disinfection of oocysts in 0·5% hypochlorite solution, Percoll-gradient (Sigma, St Louis, MO, USA) separation and Nycodenz-gradient (Sigma) purification. The purified oocysts were washed in ice-cold, sterile phosphate-buffered saline and counted with a haemocytometer. For use as ELISA/Western blot antigens, purified oocysts were treated at 37°C with 0·75% taurocholic acid for 1 h in order to release sporozoites.

Gnotobiotic piglets and serum samples

A total of 16 piglets were derived by Caesarean section and maintained under gnotobiotic conditions as described previously (19). The first group of seven 1-day-old piglets were used for oral inoculation with type 1 (TU502, pig-derived isolate) at a dose of approximately 1×10^6 oocysts per animal. The intensity of oocyst fecal shedding (0–5 scale) and clinical status of inoculated piglets were recorded daily as described (20). Blood was drawn from all seven piglets at 4 weeks of age to harvest convalescent serum. In addition, three of the TU502-inoculated piglets were intramuscularly immunized at 4 weeks of age with 5×10^7 oocysts (TU502) in Freund's incomplete adjuvant in order to produce immune sera that were harvested 3 days later. The second group of nine 1-day-old piglets was inoculated with type 2 (GCH1), and the convalescent sera were harvested similarly as described for piglets inoculated with type 1. Four piglets were assigned for production of immune sera to type 2.

Western blot

C. parvum proteins (3×10^6 excysted oocysts per lane) were separated (40 mA for 3 h) by 8–16% sodium dodecyl sulphate polyacrylamide gradient gel electrophoresis (Criterion-Precast, Bio-Rad, Hercules, CA, USA). Separated proteins were transferred to nitrocellulose membrane (395 mA, 18 h, 4°C) and blocked with 5% w/v low-fat milk in TBS-T for 1 h, 25°C to prevent nonspecific antibody binding. After blocking, the membranes were washed with TBS-T and probed with *C. parvum*-specific pig or mouse polyclonal antibodies for 2 h at 25°C. Horseradish peroxidase-conjugated goat antibodies to pig immunoglobulin (Ig)G (Bethyl-Laboratories, Montgomery, TX, USA) and to mouse IgG (Sigma) were used as secondary antibodies. A colourimetric reaction was developed, using the tetramethylbenzidine chromogenic substrate (TMB, Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA).

C. parvum recombinant gp15 and gp40 proteins and antibodies

Recombinant GCH1-derived gp15 and gp40, expressed in *Escherischia coli* pET32/Xa/LIC vector as 28 and 50 kDa fusion proteins, respectively, and generated as described previously (13), containing thioredoxin, His and S tags, were used in Western blots. In addition, negative control fusion protein expressing only thioredoxin, His and S tags and the additional eight amino acids of molecular weight 20 kDa was used. Fusion proteins were purified by metal ion-affinity chromatography using a Talon kit (Clontech, Palo Alto, CA, USA) according to the manufacturer's instructions. Protein estimation was performed using a Micro BCA kit (Pierce, Rockford, IL, USA). A total amount of 0·2 g per lane of each recombinant protein was loaded. To produce mouse polyclonal antisera to recombinant gp40 and gp15, 3-week-old BALB/c mice were immunized intraperitoneally with the respective fusion protein excised from SDS polyacrylamide gels at 2-weekly intervals until antibody production was achieved, as determined by immunoblotting of an oocyst lysate with sera obtained by tail bleeds.

Deglycosylation of *C. parvum* native proteins

C. parvum proteins were separated on 5–15% gradient SDS-PAGE gels and transferred to nitrocellulose membrane.
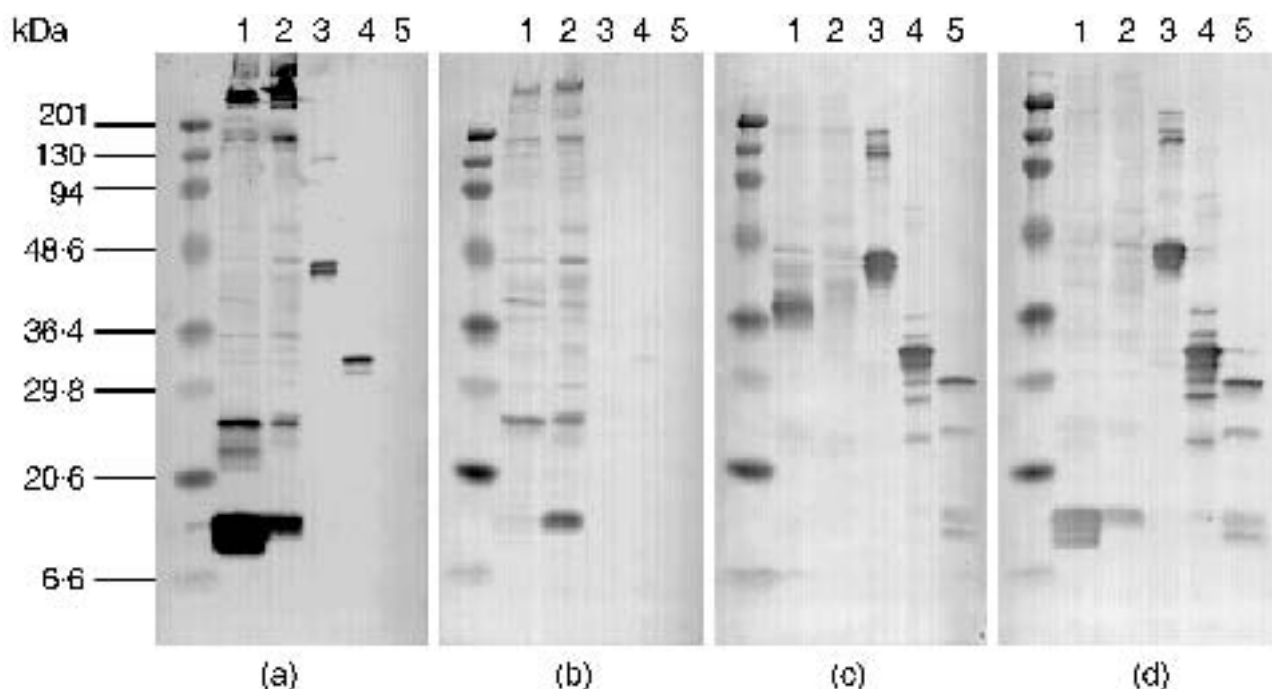
Figure 1 The *C. parvum* specific IgG seroconversion against type 1 (TU502) and type 2 (GCH1) was tested by Western blot to assess the reactivity against homologous and heterologous native and recombinant proteins. (a) Convalescent serum collected from GCH1-inoculated gnotobiotic pig at PID 25 was used in Western blot reaction with GCH1 antigens (lane 1), TU502 antigens (lane 2), gp40 recombinant protein (lane 3), gp15 recombinant protein (lane 4), or negative control fusion protein (lane 5); (b) convalescent serum collected from TU502-inoculated gnotobiotic pig at PID 28 in reaction with respective *C. parvum* antigens; (c) serum collected from GCH1-derived gp40 recombinant protein immunized mouse; and (d) serum collected from gp15 recombinant immunized mouse.

Transferred proteins were deglycosylated by periodate oxidation as described (21). Briefly, membranes were exposed to 10 mM periodate in 50 mM sodium acetate buffer (pH 4·5) for 1 h in the dark at room temperature (25°C). Membranes were then rinsed with sodium acetate buffer and incubated with 50 mM sodium borohydride in PBS for 30 min at room temperature. Treated proteins were probed with pig anti-*C. parvum* antibodies for 1 h at room temperature. Horseradish peroxidase-conjugated rabbit antipig IgG (Sigma) was used as secondary antibody. A colourimetric reaction was developed, using the tetramethylbenzidine chromogenic substrate.

Sequence analysis of *C. parvum Cpgp40/15*

The type 2 Iowa isolate *Cpgp40/15* nucleotide sequence was searched against the nonredundant GenBank database using the BLASTN algorithm. Resulting sequences (15 type 1 and 14 type 2 isolates) were sorted according to probability significance scores of homology. The GenBank accession numbers AF114166–AF224464 were used. These sequences were subjected to BLASTN searches against the GenBank database to identify clones homologous to *Cpgp40/15*

amino-acid sequence of *C. parvum* type 1 NEMC1 isolate, or to *Cpgp40/15* amino-acid sequence of *C. parvum* type 2 IOWA isolate.

RESULTS

All 16 pigs inoculated with *C. parvum* type 1 or type 2 isolates developed clinical symptoms of cryptosporidiosis characteristic of diarrhoea and oocyst shedding in faeces. The length of oocyst shedding varied between 1 and 3 weeks for both *C. parvum* isolates. The identity of *C. parvum* used for inoculation and *C. parvum* shed in faeces was confirmed by genotyping utilizing the restriction fragment length polymorphism and multilocus microsatellite analysis as previously described by our group (17). No displacement of type 1 with type 2 and vice versa was observed (not shown).

When convalescent sera from GCH1 and TU502 inoculated animals were analysed by Western blot, distinct reactivity patterns with homologous and heterologous native and recombinant proteins were found (Figure 1). Convalescent serum from GCH1 inoculated pigs (Figure 1a) showed prominent reactions with both GCH1 (lane 1) and TU502 (lane 2) native antigens, and GCH1-derived gp40 (lane 3)

and gp15 (lane 4) recombinant proteins. No reactivity with the negative control recombinant protein was observed (lane 5). In addition, immunodominant protein-band (s) positioned between 6·6 and 20·6 kDa (gp15) was detected in the case of GCH1 antigen in comparison to TU502 antigen (Figure 1a). Convalescent serum from TU502 inoculated pigs (Figure 1b) showed less or no reactivity with native gp15 of GCH1 (lane 1), gp40 recombinant (lane 3) and gp15 recombinant (lane 4), while strongly reacting with native gp15 of TU502 (lane 2). Polyclonal mouse sera from animals immunized with recombinant gp40 (Figure 1c) and gp15 of GCH1 (Figure 1d) exhibited a stronger reaction at the level of both native GCH1 than TU502 antigens (Figure 1c,d). Both recombinant proteins (gp40 and gp15) were recognized by serum generated against either protein (Figure 1c,d). The cross-reactivity of both recombinant proteins with antisera to each recombinant protein was due to the presence of antibodies to the fusion tags that were present in both fusion proteins with which the mice were immunized (Figure 1c,d). Both major (gp15) and minor (gp20–29 kDa) antigenic differences were observed (Figure 1), suggesting that gp40/15 is not the only surface glycoprotein under the host immune pressure.

The sera from pigs that had been orally inoculated and intramuscularly immunized with *C. parvum* (immune sera) were tested by Western blot (Figure 2) to confirm the results generated by convalescent sera (Figure 1). In addition to a prominent (gp15) difference in reactivity of immune serum prepared against GCH1 (Figure 2a) and TU502 (Figure 2b) antigens, minor differences were also observed (gp23). No difference was observed for gp900 (Figure 2). When GCH1 and TU502 antigens were treated by periodate oxidation, decreased/no reactivity of the three major immunodominant proteins was observed (Figure 3). From comparative analysis of published *Cpgp40/15* amino-acid sequences of 15
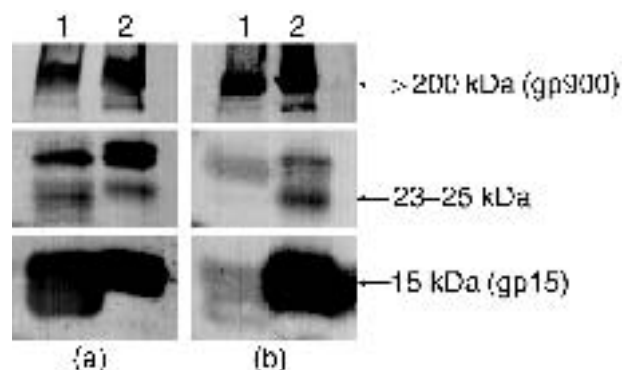

Figure 2 The immune sera from pigs inoculated orally and immunized intramuscularly with type 1 (TU502) or type 2 (GCH1) sporozoites/antigens were used for comparisons of their IgG reactivity against *C. parvum* immunodominant proteins in Western blot. (a) Immune serum from GCH1-immunized pig showed prominent reaction against gp900, gp23 and gp15. The reaction was different between GCH1 (lane 1) and TU502 (lane 2) antigens at the level of gp23 and gp15. (b) Abrogation of reaction with GCH1 gp23 and gp15 antigens was observed in the case of immune serum from TU502 immunized pig (lane 1) while prominent reaction against homologous TU502 gp900, gp23 and gp15 antigens was demonstrated (lane 2).

type 1 and 14 type 2 *C. parvum* isolates, more than 80% homology and significant similarity ($P < 0.00005$) was found (Figure 4). Whereas the type 2 homologies were 98–100%, type 1 isolates were 84·8–100% and type 1 versus type 2 isolates were 87–94·3% homologous (Figure 4).

DISCUSSION

Recently published studies on *C. parvum Cpgp40/15* surface glycoprotein gene composition and antigen characteristics
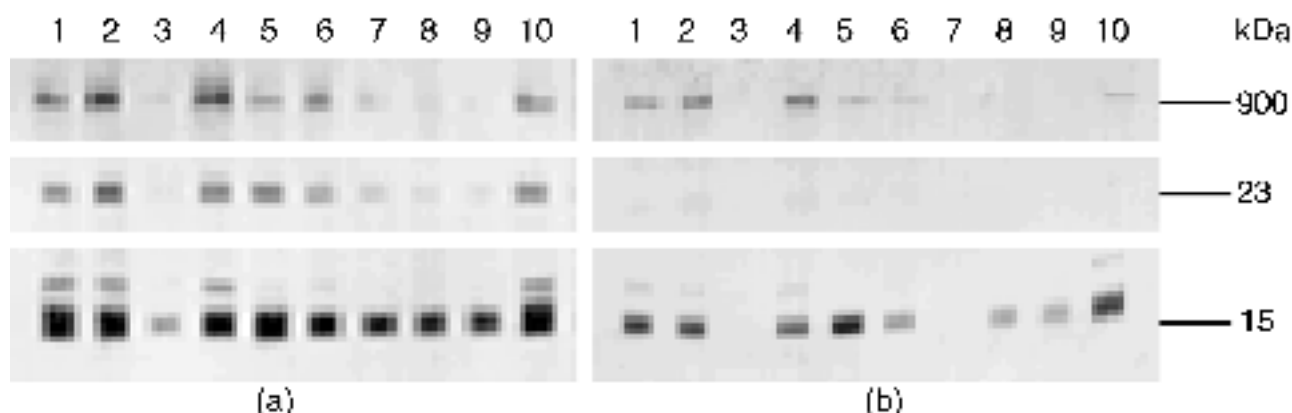

Figure 3 Untreated (a) and periodate oxidation treated (b) GCH1 antigens were tested by Western blot (IgG) against a panel of serum samples collected from pigs inoculated with GCH1 (lanes 1–7 and 10) or TU502 (lanes 8 and 9). Decreased/no reactivity of the three major immunodominant glycoproteins (gp900, gp23 and gp15) was observed after periodate oxidation treatment (b).
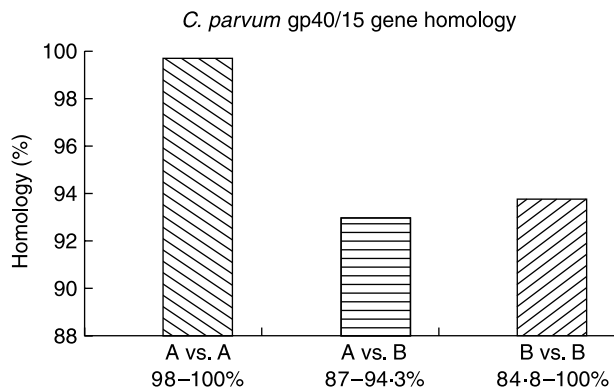
C. parvum gp40/15 gene homology

Figure 4 Summary of *C. parvum* type 1 versus type 2 gp40/15 genetic sequence similarity using the published (GenBank) sequences. Nucleotide sequences of type 2 isolates (A) exhibit a greater (98–100%) level of similarity than type 1 isolates (B) (i.e. 84·8–100%). When type 1 sequences were compared with type 2 sequences, genetic sequence similarity was 87–94·3%.

shed new light on the taxonomy and evolution of *C. parvum* (12–14). The glycosylated nature of these proteins suggests a possible role in parasite attachment and invasion of host cells (enteric epithelium) (12–16). *Cpgp40/15* genetic sequence differences among genotype 1 and 2 *C. parvum* isolates suggests that these proteins are likely targets of selective pressure applied by the host immune system. The level of *Cpgp40/15* genetic and amino acid diversity suggests that antigen differences should also occur, a feature that was investigated in our study. We used the two *C. parvum* prototype isolates: TU502 (genotype 1) and GCH1 (genotype 2), since both isolates had been adapted in our laboratory for serial *in vivo* propagation in the gnotobiotic pig model (11).

To assess the antigenicity of TU502 and GCH1 proteins, and to identify possible differences at the level of *Cpgp40/15*, we used convalescent and immune sera from inoculated/immunized gnotobiotic pigs. We also used mouse sera from animals immunized with recombinant proteins to verify the results generated with native *C. parvum* proteins. To illustrate the genetic diversity of *C. parvum Cpgp40/15*, we used published (GenBank) sequences of 15 type 1 and 14 type 2 sequences (Figure 4). As reported by Strong *et al.* (14), the *Cpgp40/15* gene is the most polymorphic *C. parvum* protein so far identified; moreover, variations can be observed between type 1 and type 2 isolates and also among type 1 but not type 2 isolates (Figure 4). It remains to be elucidated why type 2 isolates do not exhibit the same level of polymorphism as those of type 1. It was suggested that type 1 isolates could be further subdivided into four allelic subgroups based on *Cpgp40/15* genomic analysis of 29 *C. parvum* isolates (14). To assess the corresponding antigenic differences,

we used the two prototype isolates on the understanding that further antigen differences could be identified among type 1 isolates in future studies. The major antigen differences between the two isolates (TU502 and GCH1) were identified on the level of gp15 by utilizing the polyclonal antibodies against both native and recombinant gp15. The minor antigen differences between the two analysed isolates were found in the range of gp23–27. This is consistent with findings from other studies that also utilized convalescent animal or human sera and identified gp15 and gp23–27 as immunodominant (22–28). Using the protein analysis tools available in the MacVector software (Accelrys Inc., Princeton, NJ, USA) (GCG), the amino acid sequence, VEDASKRDKY (type 2 sequence), is predicted to be surface-exposed and a potential antigenic site (unpublished). This sequence is the most polymorphic region within the type 1 gp15 sequences, suggesting that this might be an important epitope.

In earlier studies, a prominent serum IgM and IgG reactivity to 15-, 17- and 27-kDa *C. parvum* proteins in experimentally infected human volunteers was reported (24). Studies on the ability of *C. parvum* specific serum and mucosal secretions to prevent infection demonstrated that increased IgA levels do not correlate with protection (29). A DNA-vaccine that encoded another *C. parvum* gene (also named gp15 but with a different nucleotide sequence to the gp15 referred to in the present study) was reported to induce protective IgG in pregnant goats, which is also transferred to offspring (30). In our study, we found that not only was gp15 the most prominent immunodominant *C. parvum* protein, but it also exhibited the distinct Western blot reactivity for GCH1 or TU502 isolates. In studies with *C. parvum* infected calves, IgM, IgG and IgA antibodies specific to *C. parvum* gp23 were present in intestinal secretions up to 2 months after inoculation (31). In our study, gp23 was among the three recognizable immunodominant proteins, together with gp15 and gp900. Although the differences between the GCH1 and TU502 were more prominent for gp15, minor differences were also observed for gp23–25. Deglycosylation treatment of immunodominant proteins (gp15, 23 and 900) with sodium periodate resulted in reduced reactivity with serum antibodies. This is in an agreement with the conclusions from previous studies showing that the structure and function of *C. parvum* surface proteins is glycosylation-dependent (12,14).

In other studies with gnotobiotic piglets inoculated with type 1 oocysts, a displacement of the original type 1 isolate to type 2 had occurred (Widmer and Tzipori, unpublished observations). It was hypothesized that by inoculating the pigs with type 1 oocysts containing a type 2 subpopulation, preferential growth of type 2 oocysts would take place within several passages *in vivo* (11). In our study, the assurance of original stock identity was achieved using the *in vivo*

adapted TU502 isolate. This isolate has been serially passaged 12 times in gnotobiotic pigs without a change in genotype as demonstrated by restriction fragment length polymorphism and multilocus microsatellite analysis (17). We contemplate that future studies on genetic and antigenic characterization of *C. parvum* isolates will identify further differences in *Cpgp40/15* with possible consequences on epidemiology, diagnosis, treatment and prevention of cryptosporidiosis.

ACKNOWLEDGEMENTS

REFERENCES

1 Newman RD, Sears CL, Moore SR, Nataro JP, Wuhib T, Agnew DA, Guerrant RL, Lima AAM. Longitudinal study of *Cryptosporidium* infection in children in Northeastern Brazil. *J Infect Dis* 1999; **180**: 167–175.

2 Griffiths JK. Human cryptosporidiosis: epidemiology, transmission, clinical disease, treatment and diagnosis. *Adv Parasitol* 1998; **40**: 37–81.

3 Tyzzer EE. A sporozoon found in the peptic glands of the common mouse. *Proc Soc Exp Biol Med* 1907; **5**: 12–13.

4 Panciera RJ, Thomassen RW, Garner FM. Cryptosporidial infection in a calf. *Vet Pathol* 1971; **8**: 479.

5 Navin TR, Hardy AM. Cryptosporidiosis in patients with AIDS. *J Infect Dis* 1987; **155**: 150.

6 Widmer G, Tzipori S, Fichetbaum CJ, Griffiths JK. Genotypic and phenotypic characterization of *Cryptosporidium parvum* isolates from people with AIDS. *J Infect Dis* 1998; **178**: 834–840.

7 Sulaiman IM, Xiao L, Yang C *et al.* Differentiating human from animal isolates of *Cryptosporidium parvum*. *Emerg Infect Dis* 1998; **4**: 681–685.

8 Patel S, Pedraza-Diaz S, McLauclin J, Casemore DP. Molecular characterization of *Cryptosporidium parvum* from two large suspected waterborne outbreaks. *Commun Dis Public Health* 1998; **1**: 231–233.

9 McLauchlin J, Pedraza-Diaz S, Amar-Hoetzeneder C, Nichols GL. Genetic characterization of *Cryptosporidium* strains from 218 patients with diarrhea diagnosed as having sporadic cryptosporidiosis. *J Clin Microbiol* 1999; **37**: 3153–3158.

10 McLauchlin J, Amar C, Pedraza-Diaz S, Nichols GL. Molecular epidemiological analysis of *Cryptosporidium spp.* in the United Kingdom: results of genotyping *Cryptosporidium spp.* in 1705 fecal samples from humans and 105 fecal samples from livestock animals. *J Clin Microbiol* 2000; **38**: 3984–3990.

11 Widmer G, Akiyoshi D, Buckholt MA *et al.* Animal propagation and genomic survey of a genotype 1 isolate of *Cryptosporidium parvum*. *Mol Biochem Parasitol* 2000; **108**: 187–197.

12 Cevallos AM, Bhat N, Verdon B *et al.* Mediation of *Cryptosporidium parvum* infection *in vitro* by mucin-like glycoproteins defined by a neutralizing monoclonal antibody. *Infect Immun* 2000; **68**: 5167–5175.

13 Cevallos AM, Zhang X, Waldor M *et al.* Molecular cloning and expression of a gene encoding *Cryptosporidium parvum* glycoproteins gp40 and gp15. *Infect Immun* 2000; **68**: 4108–4116.

14 Strong WB, Gut J, Nelson RG. Cloning and sequence analysis of a highly polymorphic *Cryptosporidium parvum* gene encoding a 60-kilodalton glycoprotein and characterization of its 15- and 45-kilodalton zoite surface antigen products. *Infect Immun* 2000; **68**: 4117–4134.

15 Priest JW, Kwon JP, Arrowood MJ, Lammie PJ. Cloning of the immunodominant 17-kDa antigen from *Cryptosporidium parvum*. *Mol Biochem Parasitol* 2000; **106**: 261–271.

16 Langer RC, Riggs MW. *Cryptosporidium parvum* apical complex glycoprotein CSL contains a sporozoite ligand for intestinal epithelial cells. *Infect Immun* 1999; **67**: 5282–5291.

17 Feng X, Rich SM, Akiyoshi D *et al.* Extensive polymorphism in *Cryptosporidium parvum* identified by multilocus microsatellite analysis. *Appl Environ Microbiol* 2000; **66**: 3344–3349.

18 Theodos CM, Sullivan KL, Griffiths JK, Tzipori S. Profiles of healing and nonhealing *Cryptosporidium parvum* infection in C57BL/6 mice with functional B and T lymphocytes: the extent of gamma interferon modulation determines the outcome of infection. *Infect Immun* 1997; **65**: 4761–4769.

19 Sestak K, Lee JH, Brisben J, Beauchemin M, Tzipori S. Tacrolimus-FK506 induced immunosuppression in gnotobiotic piglets. *Vet Immunol Immunopathol* 2000; **77**: 289–300.

20 Tzipori S, Rand W, Griffiths JK, Widmer G, Crabb J. Evaluation of an animal model for cryptosporidiosis: therapeutic efficacy of paromycin and hyperimmune bovine colostrum-immunoglobulin. *Clin Diagn Lab Immunol* 1994; **1**: 450–463.

21 Woodward MP, Young Jr, WW, Bloodgood RA. Detection of monoclonal antibodies specific for carbohydrate epitopes using periodate oxidation. *J Immunol Meth* 1985; **78**: 143–153.

22 Riggs MW, Cama VA, Leary HL Jr, Sterling CR. Bovine antibody against *Cryptosporidium parvum* elicits a circumsporozoite precipitate-like reaction and has immunotherapeutic effect against persistent cryptosporidiosis in SCID mice. *Infect Immun* 1994; **62**: 1927–1939.

23 Mead JR, Arrowood MJ, Sterling C. Antigens of *Cryptosporidium* sporozoites recognized by immune sera of infected animals and humans. *J Parasitol* 1988; **74**: 135–143.

24 Moss DM, Chappell CL, Okhuysen PC *et al.* The antibody response to 27-, 17-, and 15-kDa *Cryptosporidium* antigens following experimental infection in humans. *J Infect Dis* 1998; **178**: 827–833.

25 Ortega-Mora LM, Troncoso JM, Rojo-Vasquez FA, Gomez-Bautista M. Identification of *Cryptosporidium parvum* oocyst/sporozoite antigens recognized by infected and hyperimmune lambs. *Vet Parasitol* 1994; **53**: 159–166.

26 Peeters JE, Villacorta I, Vanopdenbosch E *et al. Cryptosporidium parvum* in calves. kinetics and immunoblot analysis of specific serum and local antibody responses (IgA, IgG, and IgM) after natural and experimental infections. *Infect Immunol* 1992; **60**: 2309–2316.

27 Reperant JM, Naciri M, Lochmann S, Tilley M, Bout DT. Major antigens of *Cryptosporidium parvum* recognised by serum antibodies from different infected animal species and man. *Vet Parasitol* 1994; **55**: 1–13.

28 Priest JW, Kwon JP, Moss DM *et al.* Detection by enzyme immunoassay of serum immunoglobulin G antibodies that recognize specific *Cryptosporidium parvum* antigens. *J Clin Microbiol* 1999; **37**: 1385–1392.

29 Kassa M, Comby E, Lemeteil D, Brasseur P, Ballet JJ. Characterization of anti-*Cryptosporidium* IgA antibodies in sera from immunocompetent individuals and HIV-infected patients. *J Protozool* 1991; **38**: 179S–180S.

30 Sagodira S, Buzoni-Gatel D, Iochman S, Naciri M, Bout D. Protection of kids against *Cryptosporidium parvum* infection after immunization of dams with CP15-DNA. *Vaccine* 1999; **17**: 2346–2355.

31 Wyatt CR, Bracket EJ, Mason PH, Savidge J, Perryman LE. Excretion patterns of mucosally delivered antibodies to p23 in *Cryptosporidium parvum* infected calves. *Vet Immunol Immunopathol* 2000; **76**: 309–317.